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Cytosolic Protein Translocation Factors Is SRP Still Unique?

Minireview

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The control of protein traffic into eukaryotic membrane-bounded organelles or across the prokaryotic plasma membrane is dependent upon each new molecule being directed to the appropriate translocation pathway. The requirement for discrete signal sequences in targeting proteins to the correct membrane has been well documented. There is now growing evidence, however, that after preproteins are released from ribosomes, they must be in a nonrigid conformation in order to be translocated across membranes (reviewed in Meyer, 1988, and Randall and Hardy, 1989). Several cytosolic proteins in different systems have been proposed to preserve such a translocation-competent conformation. These molecules may bind to loosely folded proteins (including, but not necessarily restricted to, preproteins) at a variety of sites via hydrophobic or other interactions. In this way, they may slow the folding of the bound proteins into conformations that are unsuitable for interaction with specific signal recognition and translocation proteins. These factors have been postulated to be components of a preprotein recognition and targeting apparatus, but with the exception of the signal recognition particle (SRP), no direct interaction with preprotein signal sequences or specific membrane receptors as part of the translocation reaction has been demonstrated convincingly. In addition to fulfilling these criteria for an essential component of the translocation machinery, SRP may also be unique because it appears to function exclusively during preprotein synthesis. Hence, it may bypass altogether the problem of preproteins adopting unsuitable conformations before crossing the membrane.

What Is Translocation Competence?

At present, translocation competence of a preprotein has merely been defined operationally. In several membrane systems, it correlates with a much greater sensitivity to proteases than is found in the fully mature protein, presumably an indication of a nonrigid conformation (Randall and Hardy, 1986; Eilers et al., 1988). Other experimental evidence suggests that proteins cross membranes in a more or less open state (Eilers and Schatz, 1986; Collier et al., 1988). Lacking detailed knowledge about the nature of the still putative translocation pores, we do not know what structures can be translocated across the membrane and what steric limitations are imposed. Branched and disulfide-linked polypeptides, and even oligonucleotides, can be imported into mitochondria, however, indicating that (at least in that membrane system) the passenger molecules do not need to be perfectly extended polypeptide chains (Vestweber and Schatz, 1989).

One way to view translocation competence is in terms of protein folding. Protein folding is thought to be a sequence of many intermediate steps. As a protein folds, it constantly probes the stability of different possible conformations and adopts the energetically most stable one to continue the process. Thermodynamically, the probability of the protein being in the energetically preferred state becomes correspondingly higher. In these sequential steps, the acquisition of secondary structure does not necessarily precede all tertiary interactions. Each step or local energy minimum is separated from the next on the folding pathway by an activation energy barrier that must be overcome, providing a kinetic hurdle. To proceed toward a completely folded protein, the next energy minimum must be lower in its free energy than the preceding one. In principle the protein will always be in equilibrium with preceding conformations on the folding pathway, but as folding proceeds, the probability of it being in an earlier conformational state becomes insignificant. Hence, a protein becomes progressively more "rigid" as it becomes more unlikely to unfold spontaneously. At a late point in the folding pathway for many proteins, a particularly large activation energy barrier is found that locks the protein into its final conformation and stabilizes it against thermal denaturation. Given these considerations, translocation competence probably is not attained at one specific stage of folding, but rather at any early stage in the folding pathway of a preprotein in which it remains in a state of low rigidity. Translocation competence can be lost if protein folding proceeds too far, or if folding obscures the signal sequence and thereby blocks interactions with appropriate receptor molecules.

Molecular Chaperones versus Translocation Factors

The term molecular chaperone was originally applied to factors that promote the assembly of oligomeric proteins (Hemmingsen et al., 1988). Such factors include the immunoglobulin heavy chain binding protein (BiP) in the lumen of the endoplasmic reticulum (ER), the 60 kd heat shock protein in yeast mitochondria (hsp60), the Rubisco subunit binding protein in chloroplasts, and groEL in E. coli. The concept can be generalized to include other molecules that function largely to maintain proteins in a state of low rigidity by kinetically stabilizing intermediate steps in their folding pathways. These factors, such as hsp70 and perhaps trigger factor and SecB, presumably identify and bind their ligands in a defined manner, as is suggested by the finding that BiP and hsp70 have a higher affinity for some sequences than others (Flynn et al., 1989). However, the rules that govern interactions with passenger proteins remain obscure.

In the absence of molecular chaperones, preproteins (or other proteins that need to be modified, processed, or assembled into oligomeric structures) might proceed to fold into an essentially irreversible rigid state before

necessary posttranslational events can occur. Molecular chaperones may allow a protein to remain in a state that is not terminally committed and be capable, for example, of engaging in translocation across a membrane or of proceeding to fold coordinately into oligomeric assemblies once other subunits become available. In principle, one chaperone could function broadly in protein folding and not be restricted to the folding of preproteins. In any event, chaperones may play no role in signal recognition per se, but rather may only keep the signal sequence of preproteins accessible for interaction with an appropriate receptor molecule by maintaining a nonrigid state.

A true cytosolic translocation factor, by contrast, would be expected to interact specifically with the signal sequence of preproteins and not merely aid in retarding folding. To show that a protein is a translocation factor, it is essential to prove that signal sequences mutants (preferably point mutations) which abolish the translocation of the precursor also prevent interaction with the factor. This type of analysis is important because typical in vitro assays measure the posttranslational translocation of a test protein across a membrane; thus, completely folded, malfolded, aggregated, or precipitated preproteins will not be transported, and any factor preventing these effects will be identified as a candidate translocation factor.

The jury is still out on several putative translocation factors that have been identified in bacteria and yeast, but in most cases, evidence for signal sequence binding is indirect or controversial. Of the cytosolic factors identified in E. coli, only the *secA* gene product has been suggested to interact with signal sequences on the basis of suppressor analysis of signal sequence mutations (Fikes and Bassford, 1989; Stader et al., 1989). Other genetic experiments suggest that SecA also binds to a membrane receptor. Several independent lines of evidence imply that the *secB* gene product interacts with internal portions of preMBP (Collier et al., 1988; Gannon et al., 1989). Moreover, *secB* is essential for viability only if cells are grown on rich medium (Kumamoto and Beckwith, 1985). The requirement for *secB* can be bypassed altogether if heat shock is permanently induced, which suggests that relatively nonspecific interactions can at least partially replace *secB* function (E. Altman and S. Emr, unpublished data). On the other hand, one recent study strongly suggests that SecB interacts with the signal sequence of preMBP (Watanabe and Blobel, 1989). Several experiments in this study provide evidence for reduced SecB binding to a translocation-defective mutant in which much of the signal sequence is deleted; it will be interesting to see whether more subtle mutations have similar effects. Trigger factor, another cytosolic protein in E.coli, interacts much better with proOmpA than with mature OmpA (Crooke et al., 1988a). This observation, however, could be accounted for by conformational differences, rather than by recognition of the signal sequence. One cytosolic factor in yeast, hsp70, is known to affect translocation of precursors with different destinations (Deshaies et al., 1988), implying that it does not recognize the signal sequence.

SRP: A Bona Fide Translocation Factor

In contrast to the more promiscuous chaperones, SRP functions as a matchmaker. During protein synthesis, SRP specifically recognizes signal sequences of nascent secretory or membrane proteins as they emerge from the ribosome. Cross-linking experiments have shown that the 54 kd subunit (SRP54) binds to the signal sequence (Krieg et al., 1986; Kurzchalia et al., 1986). In the absence of a signal sequence, SRP binds to ribosomes with micromolar affinity. This binding affinity is increased by three to four orders of magnitude when a signal sequence emerges (Walter et al., 1981). It follows that the affinity of isolated SRP for a signal sequence may be very low (in the millimolar range). Recognition then must depend to a large degree on the proper juxtaposition of the signal sequence binding site of SRP54 and the nascent chain on the ribosome. Indeed, specific binding of SRP to signal sequences in the absence of ribosomes has not been demonstrated, and an excess of free SRP54 does not compete for signal recognition with intact SRP (Walter and Blobel, 1983). As far as we know, SRP function relates to protein folding only in the sense that it segregates the signal sequence from the rest of the polypeptide and keeps it accessible for subsequent interactions with membrane components. Hence the posttranslational translocation-promoting activity of free SRP recently observed in yeast (Sanz and Meyer, 1988) and bacterial (Crooke et al., 1988a) in vitro systems may not be related to any actual function in mammalian systems. Given that SRP is known to be hydrophobic, these experiments may merely reemphasize that relatively nonspecific hydrophobic interactions are sufficient to retard protein folding.

A second feature of SRP that confirms its direct role in protein translocation is its ability to direct ligands to a specific membrane. The specificity of protein targeting to the membrane of the ER is achieved both by an interaction of SRP with the nascent chain and an interaction of SRP with the SRP receptor (docking protein), a heterodimeric ER membrane protein. A highly basic domain in the N-terminal half of the α subunit of the SRP receptor (SRα), the 68/72 kd SRP proteins, and SRP RNA have been suggested to participate in this interaction. Recent work has shown that the SRP receptor is a GTP binding protein and that GTP may be required for the release of SRP from the signal sequence and the ribosome upon targeting to the ER membrane (Connolly and Gilmore, 1989). Subsequently, the ribosome becomes membrane bound, and SRP and its receptor are presumed to dissociate and engage new nascent chains. The signal sequence may then be passed on to a secondary receptor in the ER membrane (signal sequence receptor), and protein translocation across the lipid bilayer proceeds through the action of a still uncharacterized protein translocation machinery. Thus, SRP and its receptor act catalytically in the targeting reaction and are not part of the final ribosome–membrane junction. So far there is no evidence that other cytosolic factors, with the possible exception of trigger factor, interact with a membrane receptor that catalyzes transfer of the polypeptide chain across the mem-

brane. It has been suggested that trigger factor interacts with SecY, an integral membrane protein, in the targeting of proOmpA (Crooke et al., 1988b; Lill et al., 1988), though it has not been shown that such interactions promote the transfer of the ligand.

SRP may also be distinct from other cytosolic factors in that its signal recognition activity may involve GTP hydrolysis. Recent work has indicated that SRP54, like SRα, contains a consensus sequence for GTP binding (Bernstein et al., 1989; Römisch et al., 1989). GTP hydrolysis has been shown to monitor or regulate the assembly of multiple components in many other processes, such as the initiation of translation. The rate of GTP hydrolysis can be used as a molecular clock. During protein synthesis, for example, an incoming tRNA must remain bound to a codon long enough for EF-Tu to hydrolyze GTP, or it will dissociate ("kinetic proofreading"). The putative involvement of GTP in signal recognition offers the intriguing possibility that GTP hydrolysis is also utilized as a timing device, perhaps to improve the fidelity of signal recognition or to slow the rate of nascent chain elongation ("translational arrest"). If SRP54 indeed monitors the quality of signal sequences, then experimental manipulations of the rate of GTP hydrolysis would affect the fidelity of signal recognition. By contrast, some molecular chaperones (e.g., hsp70, BiP) have been shown to hydrolyze ATP. In those cases, the energy of ATP hydrolysis is probably used to effect a conformational change that provides for a cycling between bound and unbound forms (Pelham, 1986) rather than to serve a regulatory role.

The determination of the cDNA sequence of SRP54 has suggested how diverse signal sequences can be recognized in a defined binding pocket in the absence of primary sequence conservation (Bernstein et al., 1989). One domain of SRP54 contains a large number of Met residues that are predicted to reside on one face of three amphipathic α helices. That these residues are of functional importance is strongly suggested by the discovery that the Met-containing helices have been strictly conserved throughout evolution: an E. coli protein that appears to be the evolutionary ancestor of SRP54 also contains Met in the same positions, and not other hydrophobic amino acids often found to be interchangeable in phylogenetic comparisons. Met side chains are unique because they are flexible whereas both Leu and Ile side chains are branched and hence comparatively rigid. The postulated α helices could form or contribute to a binding groove on the surface of the protein. The highly hydrophobic signal sequences could then interact with the Met residues and, provided they can conform to the steric constraints imposed by the groove, be accommodated by the flexible environment. Since the groove is not composed only of Met side chains, there may be certain additional contraints at local points that would result in different affinities and possibly allow discrimination among individual sequences.

In summary, SRP meets the most stringent criteria for a cytosolic factor that participates directly in protein translocation. Its function can be understood as a series of defined binding interactions in which GTP may be utilized to provide vectoriality and fidelity. Homologous proteins to SRP54 and SRα have been identified in E.coli (Bernstein et al., 1989; Römisch et al., 1989); although their functions are presently unknown, future studies will elucidate their role in this organism in which posttranslational targeting and translocation appear to be more prominent than in mammals.

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